Transcript AA454543 Is a Novel Prognostic Marker for Hepatocellular Carcinoma after Curative Partial Hepatectomy

Siu Tim Cheung*, Jenny C. Y. Ho*, Ka Ling Leung*, Xin Chen†, Daniel Y. T. Fong†, Samuel So§ and Sheung Tat Fan*

*Centre for the Study of Liver Disease and Department of Surgery, The University of Hong Kong, Pokfulam, Hong Kong, China; †Department of Biopharmaceutical Sciences, University of California-San Francisco, San Francisco, CA, USA; ‡The Clinical Trials Centre, The University of Hong Kong, Pokfulam, Hong Kong, China; §Department of Surgery, Asian Liver Center, Stanford University School of Medicine, Stanford, CA, USA

Abstract

BACKGROUND: We have previously reported on the cDNA microarray gene expression profiles of hepatocellular carcinomas (HCCs). Among the genes that show prognostic significance and are overexpressed in tumor compared with adjacent nontumorous liver, transcript AA454543 may have potential for practical use. Our aim is to validate the prognostic significance of transcript AA454543 by alternative research methods and in a separate group of HCC patients.

METHODS AND RESULTS: The data of transcript AA454543 derived from microarray analysis of 48 patients having curative partial hepatectomy (group 1) were verified by quantitative reverse transcription polymerase chain reaction (r = 0.618, P < .001). A separate sample set of HCCs obtained from 53 patients (group 2) was examined and the association of AA454543 expression level with overall survival was again validated (P = .027). By Cox regression analysis, transcript AA454543 [hazard ratio (HR) = 3.0, P = .017] and pathologic tumor node metastasis (pTNM) stage (HR = 3.3, P = .010) were independent prognostic factors for overall survival. The accuracy of prediction for 3-year overall survival for transcript AA454543 (74.2%, P = .001) and pTNM stage (76.4%, P = .001) was comparable as measured by the area under the receiver operating characteristic curve. CONCLUSION: Transcript AA454543 is a potentially useful molecular prognostic marker for overall survival after curative partial hepatectomy for HCC.


Keywords: AA454543, overall survival, liver cancer, gene expression, prognosis.

Introduction

Hepatocellular carcinoma (HCC) is prevalent in Asia and Africa and is among the five leading causes of cancer deaths worldwide [1]. Surgical resection in the form of partial hepatectomy or liver transplantation is the choice for curative treatment of this malignant disease [2–5]. Nonetheless, recurrence is still common after curative surgery and the cumulative 5-year survival rate is less than 50% for partial hepatectomy [6–9]. Thus, a prognostic marker is needed to identify patients who are at high risk of recurrence and may need aggressive adjuvant treatment. In general, pathologic tumor node metastasis (pTNM) staging accurately predicts patient long-term survival, but among patients with the same pTNM stage, a difference in long-term survival is frequently observed. As the behavior of HCC could be affected by genetic composition and expression pattern, we decided to study the gene expression difference between HCCs and their adjacent nontumor liver in relation to patient outcome after hepatectomy [10]. From our preliminary analysis of 48 patients, 1404 cDNA clones showed at least four-fold difference from the mean and, among these genes, transcript AA454543 was identified to have significance in predicting long-term survival as it ranks high in prognosis prediction and it has a high expression level in tumors relative to nontumors.

The transcript AA454543 sequence (clone ID IMAGE: 838048; UniGene Cluster Hs.437039; accession BC043195) is a 1703-bp mRNA with partial codons and was originally cloned from the hypothalamus of the human brain. By sequence homologue search with the National Center for Biotechnology Information (NCBI) BLAST, transcript AA454543 shows 95% identity over 1686 bp with AL035705, which is the human DNA sequence from clone RP4-758N20 on chromosome 1p31.3-32.2. Compared with the mouse genome, transcript AA454543 shows 85% identity over 327 bp with

Abbreviations: HCC, hepatocellular carcinoma; AFP, α-fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; pTNM, pathologic tumor node metastasis

Address all correspondence to: Dr. Siu Tim Cheung, Department of Surgery, University of Hong Kong Medical Centre, L9-55, Faculty of Medicine Building, 21 Sassoon Road, Hong Kong, China. E-mail: stcheung@hkucc.hku.hk

*This work was supported by grants from the Distinguished Research Achievement Award and the Sun Chieh Yeh Research Foundation for Hepatobiliary and Pancreatic Surgery, the University of Hong Kong.

Received 6 July 2004; Revised 24 August 2004; Accepted 1 September 2004.

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DOI 10.1593/neo.04472
AL929466, which is the DNA sequence on mouse chromosome 4. No known gene in the genomes of human, mouse, and model organisms shows a high sequence homology with the transcript AA454543 sequence.

In this study, we aimed at validation of prognostic significance of transcript AA454543 by quantitative reverse transcription polymerase chain reaction (RT-PCR) in the group of patients from whom the cDNA microarray data were derived and in a separate group of HCC patients. We also studied the significance of transcript AA454543 in patient survival in comparison with pathologic staging.

Patients and Methods

Patients and Samples

Forty-eight patients who underwent curative partial hepatectomy during the period March 1999 to April 2000 at the Queen Mary Hospital, Hong Kong were selected for the initial study (group 1). The gene expression profile of these 48 patients had been studied by cDNA microarray [10]. To validate the data obtained from cDNA microarray, in this study, quantitative RT-PCR was performed in HCCs of this group for the AA454543 expression. Another 53 HCC patients (group 2) operated during the period April 2000 to March 2002 in the same institute with the same inclusion criteria were recruited for further validation study by RT-PCR for transcript AA454543. This independent cohort of patients (group 2) was used to confirm that the prognostic marker works in general, and not only on the group of patients from whom the data are derived (group 1) [11]. Patients were included in this study if the pathologic examination of the resected specimen showed a clear resection margin. Patients were not selected if the pathologic examination showed a mixture of other tumor cell types (e.g., cholangiocarcinoma); if they had received chemotherapy before or after resection; if they had undergone liver transplantation instead of partial hepatectomy; if the resection was performed for recurrence or palliative intent; or if the resection was followed by hospital death. The clinico-pathologic data of the two groups of patients were listed in Table 1. The age of the patients ranged from 13 to 79 years, with a median age of 52 years. There were 81 men and 20 women. Serum hepatitis B surface antigen was positive in 92 patients of 101 patients succumbed to disease and the median survival period was 12.5 months (range: 4.5–34.1 months). For the remaining 70 patients, the median follow-up period was 33.4 months (range: 14.9–48.8 months).

Patients and Methods

Patients and Samples

Table 1. Clinico-Pathological Features of HCCs.

<table>
<thead>
<tr>
<th>HCC Features</th>
<th>Group 1 (n = 48)</th>
<th>Group 2 (n = 53)</th>
<th>Total n = 101</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>51 (13–73)</td>
<td>53 (16–79)</td>
<td>52 (13–79)</td>
</tr>
<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
<td>36</td>
<td>45</td>
<td>81</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>pTNM stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stages I and II</td>
<td>22</td>
<td>21</td>
<td>43</td>
</tr>
<tr>
<td>Stages III and IVa</td>
<td>26</td>
<td>32</td>
<td>58</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 5 cm</td>
<td>24</td>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>24</td>
<td>38</td>
<td>62</td>
</tr>
<tr>
<td>Venous infiltration</td>
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<td></td>
</tr>
<tr>
<td>Absence</td>
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<td>21</td>
<td>48</td>
</tr>
<tr>
<td>Presence</td>
<td>21</td>
<td>32</td>
<td>53</td>
</tr>
<tr>
<td>Microsatellite nodules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>26</td>
<td>25</td>
<td>51</td>
</tr>
<tr>
<td>Presence</td>
<td>22</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>Edmondson-Steiner</td>
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<td></td>
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<tr>
<td>Grades 1 and 2</td>
<td>20</td>
<td>23</td>
<td>43</td>
</tr>
<tr>
<td>Grades 3 and 4</td>
<td>28</td>
<td>30</td>
<td>58</td>
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<tr>
<td>Serum AFP level</td>
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<td></td>
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</tr>
<tr>
<td>≤ 20 ng/ml</td>
<td>15</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td>&gt; 20 ng/ml</td>
<td>33</td>
<td>34</td>
<td>67</td>
</tr>
<tr>
<td>HBsAg</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>43</td>
<td>49</td>
<td>92</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>4</td>
<td>9</td>
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<tr>
<td>Underlining liver disease</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Noncirrhosis</td>
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<td>64</td>
</tr>
<tr>
<td>Cirrhosis</td>
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<td>14</td>
<td>37</td>
</tr>
<tr>
<td>Disease mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>17</td>
<td>14</td>
<td>31</td>
</tr>
<tr>
<td>Alive</td>
<td>31</td>
<td>39</td>
<td>70</td>
</tr>
</tbody>
</table>

In case of uncertainty, hepatic arteriography and a post-Lipiodol computed tomography scan were performed, and, if necessary, fine-needle aspiration cytology was used for confirmation. Up to the date of analysis, 31 out of the total 101 patients succumbed to disease and the median survival period was 12.5 months (range: 4.5–34.1 months). For the remaining 70 patients, the median follow-up period was 33.4 months (range: 14.9–48.8 months).

Normal liver specimens from 30 organ donors (8 cadavric and 22 live donors) were collected in transplant operations performed at the same institution from April 2000 to December 2001 for cDNA microarray study and quantitative RT-PCR assay for transcript AA454543. The organ donors had no underlying liver diseases and were negative for hepatitis B serology. The liver specimens were obtained immediately on laparotomy to minimize the chance of DNA/RNA alteration as a result of physiologic changes or physical manipulation. Informed consents had been obtained for specimen collection. The study protocol was approved by the Ethics Committee of the University of Hong Kong.
**Microarray Expression Study**

The cDNA microarray slides were printed with about 23,000 cDNA clones including 17,400 genes. Samples, RNA preparations, and hybridization protocols had been established and described in detail previously [10, 14]. Data were deposited into the Stanford Microarray Database (http://genome-www5.stanford.edu/MicroArray/SMD) [15]. The fluorescence signals were normalized by mean-centering genes for each array and then mean-centering each gene across all arrays. Only well-measured genes were included in subsequent analyses, and defined as genes that had a ratio of signal intensity to background noise of more than 1.5-fold and net signal intensity to background of more than 50 U, for either the Cy5-labeled sample or the Cy3-labeled reference, in at least 50% of the tested samples. A total of 1404 cDNA clones, with expression levels different by at least four-fold from the mean in at least two samples, was selected for further analyses by Cox regression.

**Quantitative RT-PCR for Transcript AA454543**

Quantitative RT-PCR was performed as described [16]. Briefly, the first-strand cDNA was synthesized from 0.5 μg of total RNA using the High-Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA) following the manufacturer’s instruction. Each 25-μl PCR reaction contained 1 × PCR buffer II; 5.5 mM MgCl₂; 0.2 mM each of dATP, dCTP, and dGTP; 0.4 mM dUTP; 0.625 U of AmpliTaq Gold; and 5 μl of first-strand cDNA. Primer and probe reagents for 18S rRNA (Pre-Developed TaqMan Assay Reagents; Applied Biosystems) were used as the endogenous normalization control. Primers and probe for transcript AA454543 were AA454543-F (5′-ACC CAC ACA CAG CGC TCA C-3′), AA454543-R (5′-CAA GCC GTA AAA CTT CTG CAT G-3′), and AA454543-P (5′-6FAM AGT CAC TCT CAG CGG CCA TCG CCC A-3′). Quantification was performed using the ABI Prism 7700 sequence detection system (Applied Biosystems). Transcription quantification was performed in at least triplicates for every sample, and the threshold line was usually set at R_n = 0.1. The relative amount of transcript AA454543 had been normalized against the amount of control 18S and relative to the calibrator, and expressed as ΔΔCt, where ΔΔCt = [Ct(AA454543) – Ct(18S)] calibrator – [Ct(AA454543) – Ct(18S)] test sample. The calibrator was the sample at which the amount of transcript AA454543 was of baseline level of the sample series. The relative amount of AA454543 was presented as the relative fold difference in log 2 base scale.

**Statistical Methods**

Cox regression analyses with gene expression data as continuous variables were computed to examine gene expression that was associated with the overall survival after curative resection. The technical concern of microarray data reproducibility was addressed by using quantitative RT-PCR for validation. Correlation of expression data by microarray and quantitative RT-PCR data was assessed by the Spearman correlation test. The association of transcript AA454543 expression and overall survival was validated in group 2 patients by quantitative RT-PCR.

The overall accuracy of using transcript AA454543 expression level for prognosis prediction was measured by the area under the receiver operating characteristic curve, as there could be limitations of using hazard ratio in gauging the performance of a prognostic marker [17]. The prediction power for 3 years was analyzed. Patients who were alive but with less than 3 years of follow-up were excluded from the prediction study. Thus, 59 patients, with 31 of them succumbing to disease, were included in this part of analysis. The Youden index (sensitivity + specificity – 1) [18] was used to determine the optimal cutoff point of transcript AA454543 expression for the prediction of 3-year overall survival. The Youden index was employed to maximize the sensitivity (true-positive fraction) and specificity (1 – false-positive fraction) of the prediction simultaneously.

The association of gene expression and pTNM stage with patient outcome was examined by univariable and multivariable Cox proportional hazards regression with the forward stepwise selection procedure. pTNM stage information was categorical data. To ease interpretation, the gene expression data were modeled as categorical variable only in the multivariable Cox regression to comprehend the hazard ratios in a more interpretable scale for direct comparison with pTNM stage. The transcript AA454543 expression data were also modeled as categorical variable in the Kaplan-Meier analyses.

The associations of transcript AA454543 expression level with clinico-pathologic features were assessed by the Spearman correlation and Mann-Whitney U test, where appropriate. Differences were considered significant when P value was less than 0.05. Statistical analyses were aided by the SPSS version 11.0 software package (SPSS, Inc., Chicago, IL).

**Additional Microarray Information**

The microarray study was carried out following the MIAME guidelines issued by the Microarray Gene Expression Data Group [19]. The original data are available in the Stanford Microarray Database (http://genome-www5.stanford.edu). Information is also available from the authors on request.

**Results**

**Transcript AA454543 Expression and Overall Survival**

In the cDNA microarray data, the transcript AA454543 ranks high in prognosis prediction (top 15) and in expression level relative to nontumors (top 2) (Supplementary Table 1). Higher transcript AA454543 level by cDNA microarray was significantly associated with shorter overall survival [hazard ratio (HR) = 1.8, 95% confidence interval (CI) 1.1–3.1, P = .024] (Table 2). Quantitative RT-PCR was performed on the HCC samples of the group 1 patients to verify the cDNA microarray data. The two research methods demonstrated a high concordance (Spearman correlation, r = 0.618, P < .001). In group 2 patients, transcript AA454543 expression level as measured by quantitative RT-PCR showed a
significant association with the overall survival (HR = 1.4, 95% CI 1.0–2.0, \(P = .027\)) (Table 2).

The two independent sample sets examined by two different techniques both indicated that a higher expression level of transcript AA454543 in HCC was associated with poor overall survival after curative surgery. The two sample sets were then included into Cox regression analyses with transcript AA454543 expression level based on quantitative RT-PCR data. The transcript AA454543 level was significantly associated with overall survival in the combined data-set (HR = 1.3, 95% CI 1.1–1.6, \(P = .008\)) (Table 2).

**Prognosis by Transcript AA454543 Expression and pTNM Stage**

All patients in the two groups were included in the overall survival analyses. The transcript AA454543 expression data were based on quantitative RT-PCR method, and the prediction power for overall survival was compared with pTNM stage. The accuracy of using transcript AA454543 expression for predicting the 3-year overall survival rate was 74.2% (95% CI 61.2–87.2%, \(P = .001\)) measured by the area under the receiver operating characteristic curve (Figure 1). For comparison, the accuracy of using pTNM stage for survival prediction was 76.4% (95% CI 64.2–88.5%, \(P = .001\)). By Youden index, the optimal cutoff value of transcript AA454543 expression to segregate patients into low- or high-transcript AA454543 expression group was 7.05 (relative fold change in log 2 base). Using this cutoff value for predicting patient outcome by transcript AA454543 expression, sensitivity and specificity were 80.6% and 67.9%, respectively. When patients were dichotomized as early-stage (stages I and II) or late-stage (stages III and IVa) groups, sensitivity and specificity of prognosis prediction by pTNM stage were 80.6% and 57.1%, respectively.

The Kaplan-Meier plot was used to further examine prediction power by using the transcript AA454543 expression level alone, or together with the pTNM stage system in a total of 101 patients. Using the Youden index as cutoff, there were 43 patients in the low-transcript AA454543 expression group (range: 0–7.02), and 58 patients in the high-transcript AA454543 expression group (range: 7.08–11.50). By using the transcript AA454543 level alone to segregate the patients, the cumulative 3-year overall survivals for patients with low- and high-transcript AA454543 levels were 86.0% (37/43) and 56.9% (33/58), respectively (log-rank test, \(P = .001\)) (Figure 2). The analysis was repeated based on the transcript AA454543 level and pTNM stages of the patients. The cumulative 3-year overall survivals was 96% (24/25) for early-stage (stages I and II) patients with a low transcript AA454543 level, 72.2% (13/18) for early-stage patients with high transcript AA454543, 72.2% (13/18) for late-stage (stages III and IVa) patients with low transcript AA454543, and 50.0% (20/40) for late-stage patients with high transcript AA454543 (log-rank test, \(P = .014\)).

By Cox regression analysis, transcript AA454543 expression data modeled as continuous variable were significantly associated with the overall survival (Table 2). However, the hazard ratios expressed in their natural scale illustrated only the change in the risk of disease-related mortality associated with a change of 1 U on the expression scale—a change too small to be understood easily. To assist interpretation, the gene expression data were modeled as categorical variable to comprehend the hazard ratios in a more interpretable scale (Table 3). The patients were segregated into low- and high-transcript AA454543 expression groups similarly as in the Kaplan-Meier analyses, using the Youden index to determine the optimal cutoff value. The data were compared with pTNM stage and liver cirrhosis (as the majority of the patients was HBV-related, where liver cirrhosis might affect patient outcome after hepatectomy). By univariable Cox regression analysis, transcript AA454543 expression (HR = 3.9, 95% CI 1.6–9.6, \(P = .003\)) and late pTNM stage (HR = 4.2, 95% CI 1.7–10.3, \(P = .002\)) were significantly associated with the overall survival, but liver cirrhosis showed no association with overall survival (\(P = .709\)). By multivariable Cox regression analysis, only transcript AA454543 expression (HR = 3.0, 95% CI 1.2–7.5, \(P = .017\)) and late pTNM stage (HR = 3.3, 95% CI 1.3–8.2, \(P = .010\)) were independent prognostic factors for overall survival.

**Transcript AA454543 Level in Liver Tissues**

Transcript AA454543 expression was higher in the HCC tissues compared to the nontumor liver tissues adjacent to.

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Table 2. Cox Regression Analyses for Overall Survival on Transcript AA454543 Expression.*

<table>
<thead>
<tr>
<th>Patients</th>
<th>n</th>
<th>HR</th>
<th>(95% CI)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>48</td>
<td>1.8</td>
<td>(1.1–3.1)</td>
<td>.024</td>
</tr>
<tr>
<td>Group 2</td>
<td>53</td>
<td>1.4</td>
<td>(1.0–2.0)</td>
<td>.027</td>
</tr>
<tr>
<td>Groups 1 and 2</td>
<td>101</td>
<td>1.3</td>
<td>(1.1–1.6)</td>
<td>.008</td>
</tr>
</tbody>
</table>

*The transcript AA454543 expression data were modeled as continuous variable. The expression data were based on the microarray data in group 1 patients, and quantitative RT-PCR in group 2 patients and the combined groups of patients.

Figure 1. The accuracy of prediction for overall survival was measured by the area under the receiver operating characteristic curve. The “sensitivity” (true-positive fraction) against “1 – specificity” (false-positive fraction) was plotted for transcript AA454543 expression level (range: 0–11.50) and pTNM stage (I, II, III, and IVa), respectively.
HCCs in the earlier observation based on the cDNA microarray approach. To validate the observation, we randomly examined 93 (out of a total of 101) liver tissues adjacent to HCCs using real-time quantitative RT-PCR to measure the transcript levels. The results indicated that the HCCs demonstrated a significantly higher transcript AA454543 level (median: 7.21, range: 0–11.50) compared to that of liver tissues adjacent to HCCs (median: 5.54, range: 1.26–10.13) (P < .001).

The higher expression level in HCCs than the liver tissues adjacent to HCCs could be interpreted as either transcript AA454543 upregulation in HCCs or transcript AA454543 downregulation in liver tissues adjacent to HCCs. To distinguish the two situations, 30 normal liver tissues were examined. In normal livers, the transcript AA454543 transcript was found to express at a low level (median: 5.31, range: 0–7.36), which was significantly lower than the HCCs (P < .001) but not significantly different from the liver tissues adjacent to HCCs (P = .382) (Figure 3).

Transcript AA454543 Expression and Clinico-Pathologic Features

To better understand the significance of transcript AA454543 expression, we analysed the association of transcript AA454543 expression level with the clinico-pathologic parameters of the HCC patients. The upregulation of transcript AA454543 expression in tumor was significantly associated with late pTNM stage (r = 0.299, P = .002), venous infiltration (P < .001), microsatellite nodules (P = .016), and high Edmondson-Steiner histologic grade (r = 0.276, P = .005). The transcript AA454543 expression level in the tumor was not significantly associated with tumor size, gender, age, HBsAg positivity, serum AFP level, or liver cirrhosis.

The AA454543 expression level in nontumor liver tissues was not significantly associated with pTNM stage, venous infiltration, microsatellite nodules, Edmondson-Steiner histologic grade, tumor size, gender, age, HBsAg positivity, serum AFP level, or liver cirrhosis.
Survival in HCV-related HCCs. The transcript AA454543 expression level can predict overall survival. The aim of the study is to consolidate the significance of the prognostic genes with the assay method, quantitative RT-PCR, which is a technique that is readily available in routine laboratories for practical use. In the current study, we reported the prognostic significance of transcript AA454543 whose expression level can predict survival for HCC patients after curative hepatectomy. Transcript AA454543 expression and pTNM stage were independent prognostic factors for overall survival by multivariable Cox regression analyses. Gene expression data, together with pTNM stage, can help to provide a more accurate overall survival prediction as illustrated in the Kaplan-Meier analyses (Figure 2).

With the microarray approach, we and the others have reported the expression profiles of HCCs [10,14,20–25]. Expression of α-fetoprotein (AFP), cell cycle regulators, genes associated with metabolism, and tumor dedifferentiation status was associated with the molecular subtypes of HCCs [10,14,20–25]. However, there have been few HCC reports on the association of gene expressions with patient outcomes. Notably, Iizuka et al. [26] reported the correlation of gene expression profile with early intrahepatic recurrence. There are fundamental differences between the present study and that of the Iizuka et al. report. In that most of the patients in Iizuka et al.’s study were HCV-related (22/33, 66.7%), whereas the patients in the present study were mostly HBV-related (92/101, 91.1% in the present cohort). Different etiological agents may have involved different carcinogenesis pathways, resulting in different molecular compositions and behaviors. Furthermore, we used the overall survival of 3 years as end-point, whereas Iizuka et al. used intrahepatic recurrence in the first year as the clinical end-point for prognosis prediction. The prognostic genes may be different for prognosis of disease recurrence and overall survival. Nonetheless, we had explored the original data set by Iizuka et al. (http://surgery2.med.yamaguchi-u.ac.jp/research/DNACHip/) and transcript AA454543 was not on the probe set list. It would thus be important to evaluate if the transcript AA454543 expression level can predict overall survival in HCV-related HCCs.

The transcript AA454543 has not been well characterized and the biologic function is unknown. Notably, a higher transcript AA454543 expression level in HCC was associated with poor prognosis and aggressive tumor features including late pTNM stage, venous infiltration, microsatellite nodules, and high Edmondson-Steiner grade (poor cellular differentiation). All these features were risk factors for poor prognosis [27,28]. Importantly, venous infiltration (presence of tumor cells in the vascular space) and microsatellite nodules were features of tumor spread [27]. Thus, the present data highly suggested the biologic role of transcript AA454543 in tumor cell invasion: an increased level of transcript AA454543 might enhance the invasive ability of the tumor cells, subsequently resulting in venous infiltration and formation of microsatellite nodules. In the hierarchical clustering analysis, transcript AA454543 was found to cluster closely with the proliferation cluster, tightly with G-protein–coupled receptor and zinc finger protein (Supplementary Figure 1), which play an important role in coordinating cell cycle progression. These genes that coexpressed with transcript AA454543 may also help to provide a hint of the transcript AA454543 function. Further studies, for example, transfection experiments to modulate transcript AA454543 level and examine its effect on tumor cell invasion are needed to elucidate the exact biologic role of AA454543 in disease progression. Nonetheless, numerous functional elements including regulatory elements are non–protein-coding in the human genome, and further bioinformatic analysis may also help to understand the biologic significance [29–31].

The association of high-level transcript AA454543 with tumor invasion further suggested that its prognostic information may not only be limited to overall survival, but may also be predictive for recurrence-free survival. The association of AA454543 expression with recurrence-free survival was also analyzed with Cox regression analysis (Supplementary Tables 2 and 3). In the combined sample set (101 HCCs), AA454543 expression was in fact significantly associated with recurrence-free survival when the expression data were modeled as continuous variables ($P = .011$) and categorical variables ($P = .018$). However, independent analyses on the two sample sets were inconclusive for the association of AA454543 expression with recurrence-free survival, where significant association was observed in group 1 patients ($P = .007$) but not in group 2 patients. The discrepancy might be due to the difference in recruitment time as group 2 patients had a significantly shorter follow-up period. Furthermore, the smaller patient number in the subgroup analysis also made it difficult to achieve statistical significance. The association of AA454543 with recurrence-free survival was not definite with the present data, and therefore we intended not to draw a conclusion at the current stage.

In the clinical samples, the transcript AA454543 level was significantly higher in HCCs compared to the parallel liver tissues adjacent to HCCs, and to normal livers. The transcript AA454543 level is informative in differentiating if the liver tissue is neoplastic, in addition to providing prognostic information. Preliminary in situ hybridization analysis on the
cell origin of transcript AA454543 indicated that a cytoplasmic signal was observed in the neoplastic hepatocytes in HCC tissue (Supplementary Figure 2), which further highlighted the potential of transcript AA454543 as a tumor marker and therapeutic target in addition to the role as a prognostic marker.

The present study indicates that the transcript AA454543 expression level can predict overall survival of patients after curative partial hepatectomy. The current approach demonstrates the power of expression profiles to identify prognostic markers feasible for clinical application. It further consolidates the prospect for considering unknown genes, instead of only focusing on well-known genes with recognized biological contribution in carcinogenesis. Prognosis by genes of unknown biologic roles has also been described in breast, prostate, and lung cancers, and central nervous system embryonal tumor [32–37]. This molecular marker provides prognostic information in general (two independent cohort of patients) and the prediction is independent of assay method (microarray or quantitative RT-PCR). By quantitative RT-PCR, this gene is feasible for routine laboratory assay. Together with pTNM stage, it could help to improve prognosis prediction and disease management for patient benefit.

Acknowledgements

We are grateful to the members of the Division of Hepatobiology and Pancreatic Surgery at the University of Hong Kong; and members of the Patrick Brown Laboratory in the Department of Biochemistry, Stanford Microarray Database, and Stanford Asian Liver Center at Stanford University for their support.

References


